Female promiscuity and genetic diversity in passerine birds

By

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PREFACE

I want to thank my supervisor, Jan Lifjeld, for trusting me with your research ideas. You are seemingly available and working 20 hours per day, and always up for a spirited discussion. Thanks for being supportive and for having a sense of humour. You have taught me many things, not all pertaining to birds and evolution.

Thanks to my co-supervisor Arild Johnsen and senior colleagues Erica Leder, Tomáš Albrecht, Eduardo Garcia-del-Rey and Magnus Popp. I've have had very educational and entertaining discussions with all of you.

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I am grateful that my parents have always been very supportive. Thanks to mom for not being very supportive when I wanted to go to art school at sixteen; what would have become of me?

My dear daughter Petra, you have being thoroughly awesome and easygoing (by baby-standards) since you've entered my life. I've yet to come home from work without you greeting me with a grin; on your better days you even throw in some arm-flailing.

Last but not least, I want to thank my wife Inger Maren. I'm just following in your footsteps, so I actually expected you to be understanding of the toils involved in finishing up a PhD. Your level of support and help, however, was far beyond what I expected. Thank you for being a superb mother and a great (unofficial) statistical supervisor.

"Unfortunately, most of today's women resemble bowerbirds that force suitors to build elaborate nests of twigs, leaves, and discarded garbage before choosing a mate. Any male who doesn't meet her standards doesn't get to mate that year; one assumes he just stays in his bower, reads bower manuals, and watches bowerbird porn."

— Stephen Colbert, I am America

LIST OF PAPERS

I. **Gohli J**, Anmarkrud JA, Johnsen A, Kleven O, Borge T & Lifjeld, JT. 2013. Female promiscuity is positively associated with neutral and selected genetic diversity in passerine birds. Evolution. 67(5):1406-1419

II. Spurgin LG. 2013. Comment on Gohli et al. (2013): "Does promiscuity explain differences in levels of genetic diversity across passerine birds?" Evolution. 67(10):3071–3072

Lifjeld JT, **Gohli J**, Johnsen A. 2013. Promiscuity, sexual selection, and genetic diversity: A reply to Spurgin. Evolution. 67(10):3073-3074

III. **Gohli J**, Lifjeld JT & Albrecht T. Migration distance is positively associated with sex-linked genetic diversity in passerine birds. Manuscript.

IV. **Gohli J**, Leder E, Garcia-del-Rey E, Johannessen LE, Johnsen A, Laskemoen T, Popp M, Lifjeld JT. Resolution of an enigmatic avian island radiation by genome-wide marker analyses. Manuscript.

V. **Gohli J**, Johnsen A & Lifjeld JT. Female promiscuity in passerine birds is dependent on phylogeny and associated with male parental care and diet. Manuscript.

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ABSTRACT

This dissertation is about female promiscuity in passerine birds. By using comparative analytical approaches, I have tried to determine why there is such variation in the frequency of this behaviour. I have found that promiscuous species/populations have higher genetic diversity both at neutral loci and at loci directly involved in the recognition of pathogens. These observations may point to benefits from heterozygosity-fitness correlation or increased immunocompetence in offspring. The correlation between female promiscuity and genetic diversity may be driven by confounding variables. I evaluate one such potential confounding variable (covariate of female promiscuity), namely migration distance, but found that female promiscuity and migration distance correlates with different types of genetic diversity (autosomal and Z-linked intronic diversity respectively). Female promiscuity is a mating strategy that varies greatly in frequency in passerines. I found that the level of female promiscuity seem to differ among closely related populations of *Cyanistes* tits, and that these differences were independent of phylogeny. The hypothesis that female promiscuity predicts sperm lengths was not supported for this *Cyanistes* dataset. At a broader phylogenetic level (95 passerine spp., 27 families), I observed substantial variation in female promiscuity. In this larger dataset I identify a strong phylogenetic signal, which means that closely related species are similar in their frequency of female promiscuity. Hence, a substantial proportion of variation in female promiscuity will lie among families of Passeriformes. Variation at this phylogenetic level is likely associated with fundamental differences in ecology, while differences among species are likely related to genetic factors. Variation in female promiscuity could also be explained by genetic diversity being more or less beneficial for different species. Using the dataset of 95 spp., I evaluate the hypothesis that parasite pressure selects for increased promiscuity, because female promiscuity may increase immunocompetence in young. I found no evidence for a direct link between parasites and female promiscuity, but the proportion of animal matter in diet (a potential proxy for parasite exposure) was positively correlated with female promiscuity. An alternate evolutionary scenario that may explain the variation in female promiscuity is one where the benefits associated with promiscuous behaviour are universal, but the extent to which the behaviour is practiced is constrained by some factor. I describe a significant negative relationship between male parental care and female promiscuity and I discuss whether it may function as a constraint of female

promiscuity. Given its link to genetic diversity, female promiscuity may be of importance for the adaptability and viability of populations. Thus, a thorough understanding of this phenomenon is interesting, not only to evolutionary biologists and ecologist, but also in conservation.

INTRODUCTION

Sexual reproduction – if you partake, you'll have a bad time

Reproduction is a source of conflict between individuals in most sexually reproducing populations. If the optimal reproductive strategies of two mating individuals are not in perfect harmony, there will be some level of sexual antagonism (Parker 2006). For instance, if a male may father more young by investing less in parental care and seeking out several partners, while the female is dependent on the male's contribution to ensure that as many young as possible reach reproductive age, there will be sexual antagonism. Likewise, if it is beneficial for the female to have additional matings with males other than the social male, which reduces the social male's paternity in the brood, we have sexual antagonism. Another venue for conflict is in reproductive competitions, which occur when members of the same sex compete over resources that are related to reproductive output. Females may fight over access to the highest quality males (Gwynne 1991) or may fight to hold several "husbands" (Oring and Lank 1982). Similarly and more commonly (Gwynne 1991), males will fight over access to or fertilization of females. Fighting may be in the literal sense, as in harem building species such as the red deer (Clutton-Brock *et al.* 1982), through sexual sneaker behaviour as in coho salmon (Gross 1996), infanticide in lions (Pusey and Packer 1994; which is also very much an example of sexual antagonism), or through several males copulating with the same female, which results in sperm competition (Parker 1970). Sexual reproduction may seem less inviting given such conflicts, and to add insult to injury, asexually reproducing animals will produce twice the number of offspring compared to sexually reproducing animals (Lodé 2012).

So why do animals reproduce sexually given this costliness and potential for unpleasantness? Many would say that the answer is genetic diversity (Bengtsson 2003; Bernstein and Bernstein 2010). While asexually reproducing animals rely only on mutations and horizontal gene transfer in making changes to the population's DNA (Vos 2009), sexually reproducing animals add meiosis and recombination to the mix (Lodé 2012), which has the potential for producing an astronomical number of different offspring genotypes. Why genetic diversity is important is a more contentious question (Aquadro 1992), but it is strongly correlated with fitness (Reed and Frankham 2003). Genetic diversity may be important because it enables adaptation to changing environments, and low levels of genetic diversity may result in inbreeding depression (Reed and Frankham 2003; Frankham 2005). An alternate

explanation for why sexual reproductions' continuous reshuffling of alleles is advantageous lies in the "Red Queen hypothesis", which states that genomes needs to be moving targets, to which pathogens may never perfectly adapt (Hamilton *et al.* 1990).

Female promiscuity

Female promiscuity occurs when females mate with multiple males, which is termed extra-pair copulations when the female is in a social bond with a male. Female promiscuity, as used here, does *not* entail indiscriminate mating, but simply extra-pair copulations. I request that the reader do not anthropomorphize or politicize my use of the term (Elgar *et al.* 2013). Female promiscuity is not the same as polyandry, where females pair-bond with several males, but is rather where females seek out males solely for the purpose of copulating with them. Until only a few decades ago, when molecular studies revealed that the social males often lost paternity to other males (Morell 1998; Griffith *et al.* 2002), sexual monogamy was considered the norm (Lack 1968). That this discovery of widespread sexual infidelity came as a surprise likely reflects that extra-pair copulations are done in such a discrete manner that it was simply not observed by researchers. As one would expect, female promiscuity produces strong sexual antagonism by forcing the social male into reproductive competition with other males. This reproductive competition often manifests through sperm competition, where sperm from different males compete over fertilization (Parker 1970).

Social monogamy is not uncommon in the animal kingdom (Morell 1998), but birds are in a league of their own with approximately 90% of species being socially monogamous (Lack 1968; Wan *et al.* 2013). As tranquil as this may sound, female promiscuity occurs in over 90% of these seemingly monogamous species (Griffith *et al.* 2002). The larges avian order, Passeriformes, is exceptionally variable with regard to the amount of female promiscuity, as measured by the number of offspring fathered by extra-pair males (Westneat and Sherman 1997). Passeriformes includes both completely sexually monogamous species (Kleven *et al.* 2008a) and the species with the highest recorded frequency of illegitimate offspring among all bird species (Mulder *et al.* 1994).

There are two important questions in the field of female promiscuity that are not fully resolved (Griffith *et al.* 2002; Wan *et al.* 2013), namely 1) what are the benefits that maintain female promiscuity (why it evolved) and 2) why there is such a large degree of variation in female promiscuity. Even though these questions have been extensively studied in many

intraspecific studies and multispecies comparative studies (Griffith *et al.* 2002; Wan *et al.* 2013), research on female promiscuity in birds is still a very active field, with numerous single-species studies published in later years. Many of these studies are evaluating the hypotheses summarized in a landmark review paper published over ten years ago (Griffith *et al.* 2002), showing that, although much energy is invested in this field, there is still confusion as to what the most important constraints, causes and benefits of female promiscuity are. Some of the proposed benefits associated with female promiscuity are that extra-pair copulations enable the choice of good genes (Møller 1988), that females receive direct benefits from mated males (Burke *et al.* 1989), and that females can choose the most genetically compatible male (Kempenaers *et al.* 1999). Also, extra-pair copulations may let the female guard herself against infertility in the social male (Sheldon 1994). The majority of the work in recent years has focused on the genetic benefits of female promiscuity, with some studies discussing how such genetic benefits may be tied to better immunocompetence in offspring (Johnsen *et al.* 2000; Richardson *et al.* 2005; Brouwer *et al.* 2010; Promerová *et al.* 2011; Arct *et al.* 2013; Dunn *et al.* 2013). However, identifying benefits to female promiscuity does not necessarily explain why different species exhibit different levels of female promiscuity. The substantial variation in female promiscuity among populations must stem from either differences in how beneficial female promiscuity is, or from differences in the strength of constraints. There are several factors that can function as constraints on female promiscuity. In response to extra-pair copulations, the social male may choose to reduce his level of parental care, which constitutes a loss of resources for the female (Mauck *et al.* 1999). Additionally, there is the increased risk of getting infected with sexually transmitted diseases and the time expended on seeking out males and partaking in extra-pair copulations. The sexual antagonism resulting from female promiscuity may also select for mate guarding behaviour in males (Johnsen *et al.* 1998), who instead could have spent their resources on behaviour that benefits the female, such as nest building or territory defence. It has been shown that bird species where female promiscuity is frequent generally have "fast" life histories (large clutch sizes, high fecundity and high mortality). Both life history (Arnold and Owens 2002) and male parental care (Arnold and Owens 2002; Matysioková and Remeš 2013) have been shown to predict the level of female promiscuity and are both potential constraints on female promiscuity.

My contribution

I have used comparative approaches in trying to understand why there is such a high degree of variation in female promiscuity among passerine birds. In my analyses, I have used both the proportion of extra-pair young (EPY) and the coefficient of among-male variance in sperm length (CV_{bm}) as proxies for female promiscuity. CV_{bm} is expected to decrease with increasing female promiscuity (Kleven *et al.* 2008b), and correlates with EPY (figure 1; Lifjeld *et al.* 2010). I wished to investigate whether female promiscuity is associated with genetic diversity on both neutral loci and on immunity genes, the later of which would point to a relationship between pathogens and female promiscuity. Petrie *et al.* (1998) found that genetic diversity was correlated with female promiscuity at the population level, and proposed that genetic diversity predicted the level of female promiscuity. This proposed causality was based on the idea that the potential for gaining genetic benefits through extra-pair copulations is determined by variation in the genetic quality of males in the population. However, increased genetic diversity through extra-pair copulations may stem from females choosing genetically dissimilar males (or gametes; Tregenza and Wedell 2000), which is expected to be beneficial with respect to producing genetically variable offspring (Williams 1975; Westneat *et al.* 1990), and has been shown to correlate with increase fitness of individual offspring (Foerster *et al.* 2003; Fossøy *et al.* 2008; García-Navas *et al.* 2009; Olano-Marin *et al.* 2011). Based on this, I have adopted a different causal view to that of Petrie *et al.* (1998), where female promiscuity is a mechanism for maintaining or increasing genetic diversity. I tested the prediction that genetic diversity increases with female promiscuity using a comparative analytical approach, which has the fundamental problem of potential hidden confounding variables that may in fact be the driver of an observed correlation. Hence, I wanted to rule out a described covariate of female promiscuity, namely seasonal migration. Further, I aimed to evaluate whether female promiscuity (CV_{bm}) varied

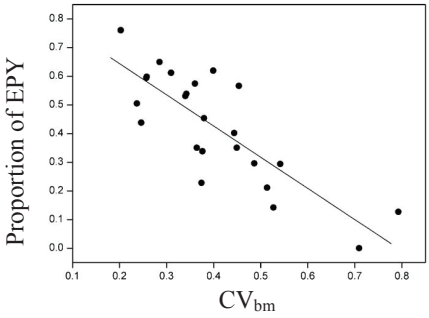


Figure 1 - The coefficient of among-male variance in sperm length (CV_{bm}) plotted against proportion of extra-pair young Lifjeld *et al*. (2011

among closely related populations in two sister species, and whether variance in female promiscuity at this level of classification was constricted by phylogenetic relationships. Lastly, I wished to analyze female promiscuity in a wider phylogenetic context, including several passerine families. In order to get an understanding of how variation in female promiscuity was distributed among different levels of classification I evaluated the strength of phylogenetic dependency in the data. Using this larger dataset, I tested predictions of the hypotheses that female promiscuity is driven by a red queen dynamic, i.e. that female promiscuity enables disassortative mating and selects rare alleles which improve immunocompetence. I also evaluated two previously described predictors/constraints of female promiscuity that have not before been tested in a strictly passerine dataset, namely life history and male parental care, both of which may function as constraints of female promiscuity.

The papers – goals and hypotheses

Paper I:

Both "good genes" and "compatible genes" have been proposed as targets of female promiscuity. By evaluating the relationship between female promiscuity and genetic diversity we can distinguish between these hypotheses, since "good genes" reduces, while "compatible genes" retains genetic diversity. We predicted that, in line with a hypothesis of "compatible genes", both selectively neutral intron sequences and number of alleles at the major histocompatibility complex (MHC) would be positively associated with female promiscuity. We wanted to test for a positive relationship between female promiscuity and the MHC loci specifically involved in pathogen recognition, which would indicate that pathogens select for more promiscuous behaviour. The MHC sequencing and principal component analyses included in this paper were done by Dr. J. A. Anmarksrud and were included in his dissertation as an unpublished manuscript.

Paper II:

Paper I received a comment in the journal Evolution. Paper II is an answer to that comment.

Paper III:

It has been proposed that seasonal migration is a strong covariate of female promiscuity, because migratory birds breed more synchronously which increases the availability of extra-pair males. In light of this it seemed prudent to reanalyze the genetic diversity data from Paper I in a multi-predictor model scheme, where we evaluated female promiscuity and migration distance and their relative abilities in explaining variation in genetic diversity.

Paper IV:

In Paper IV we wanted to test whether female promiscuity (CV_{bm}) varies among closely related populations (subspecies) of the African (*Cyanistes teneriffae*) and European blue tit (*Cyanistes caeruleus*) and whether differences in female promiscuity among populations are predicted by phylogenetic relationships, i.e. that closely related population pairs have more similar levels of female promiscuity than distantly related population pairs. We also wanted to test the prediction that the level of female promiscuity is associated to sperm length. In order to test for phylogenetic dependency in female promiscuity, we needed a trustworthy phylogeny. The phylogenetic relationships in our study-group has been studied extensively, but with inconsistent results (Grant 1979; Kvist *et al.* 2005; Dietzen *et al.* 2008; Päckert *et al.* 2013). Because there is no consensus phylogeny for our study system available, we attempted to construct a phylogeny from a next-gen RAD tag data.

The phylogeny inferred from RAD tag SNPs (single nucleotide polymorphisms) in Paper IV was surprising and suggested that previously published phylogenies might suffer from the effect of mithocondrial introgression. Because of this interesting result, the new phylogeny became the main focus when we wrote the manuscript (Paper IV) aiming for publication in a high impact journal. The reader should note that the initial questions outlined in the previous paragraph are dealt with in this introduction/thesis (Results and Discussion section), where some analyses and results that were omitted from the manuscript are presented and discussed.

Paper V:

In the fifth and last paper we aimed to evaluate several potential covariates of female promiscuity using a comparative approach. We wanted to quantify the level of phylogenetic dependency of female promiscuity in a dataset including several passerine families, and

discuss how variation in female promiscuity is distributed over different levels of classification. It has been demonstrated that life history is important in explaining variation in female promiscuity that is nested deep in the avian phylogeny. Passerines are quite homogenous in their life history traits when compared to the differences seen between orders, but there are differences among families. We will test whether these relatively small differences are related to female promiscuity. Further, we aimed to test the hypothesis that male care predicts female promiscuity. The causality for such an association is not straight forward, and will be discussed. Lastly, we wanted to test the pathogen-mediated selection hypothesis postulated in Paper I, which predicts that pathogens will select for increased female promiscuity, which improves immunocompetence. We do this by evaluating whether female promiscuity is associated with 1) a proxy of parasite exposure (foraging ecology) and 2) parasite species richness estimates (helminths and haemosporidians).

GENERAL DISCUSSION OF METHODOLOGY

The problem of causality in comparative analyses

If one is interested in whether variation in a trait is due to differences in another trait, the comparative approach can be used to test for correlations between the two. Although tests of more complex relationships are often appropriate, I will focus on analyses of linear relationships. In a regression analysis, we define a predictor variable and hypothesize that an increase in this variable will result in either an increase of decrease in a response variable. We can, for example, hypothesize that food availability will affect nestling survival in blue tits. To test this hypothesis we could collect yearly data on food availably and nestling survival over several years. A generalized least square model will trace a straight line (the model) through our data (figure 2), in such a way that the average vertical distance from the line to the data points are minimized (Hosmer and Lemeshow 2005). The test will tell us how strong the association is (β), how much of the variance in the response variable that is explained by the predictor (R^2) and whether the association is statistically significant (p-value). This may all seems very straight forward, but there is a fundamental problem with this type of analytical approach. A strong statistical association between a predictor and a response variable does not necessarily mean that changes in the response variable are caused by the predictor. They may both be governed by a third variable, a confounding variable. If we identified a strong positive relationship between food availability and nestling survival in our blue tit example, we could simply have left the matter there. It is, however, quite possible that both food availability and nestling survival is controlled by temperature (confounding variable). In our blue tit example, it seems unlikely that nestling survival will cause (measurable) changes in food availability, but in some cases researchers will mistakenly define a response variable as predictor and vice versa. The problem of not considering both causal directions and excluding potential confounding variables may lead researchers to reach the wrong conclusions. Both tests of collinearity (a linear relationship between two predictor variables) and including multiple predictors in analytical schemes are ways of dealing with the problem of confounding variables, but sadly,

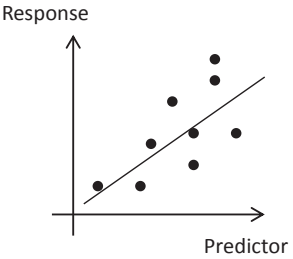
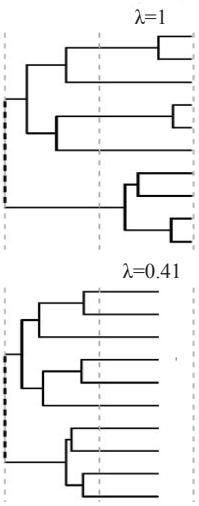


Figure 2 – Example of a linear model traced through a scatter-plot. The model estimates the covariance between the predictor and response variable.

there is no way of knowing that all possibilities have been exhausted. Careful consideration during the design of hypotheses, data collection and designing analytical schemes is of paramount importance.

Controlling for phylogeny in comparative studies

Phylogenetic dependence, i.e. the tendency of closely related taxa to be similar to one another, is an ever-present and potentially confounding variable in biological comparative analyses at the species level (Harvey and Pagel 1991). That two species of Sylviidae warblers should be more biologically and ecologically similar to each other than for instance a Sylviidae warbler and a magpie is an intuitively attractive argument. However, if a predictor variable that is different in the two Sylviidae warblers exerts selective pressure on the trait we are interested in (response variable) the two species will be dissimilar regardless of their close phylogenetic relationship (phylogenetic independence). When both the predictor and response variables are similar in closely related species matters become complicated. It is not possible to say whether it is the predictor variable that causes the response variable to be similar or if their relatedness causes them to be similar in both predictor and response. Phylogeny is always a potentially important confounding variable in evolutionary comparative analyses, but there are ways of dealing with it.



Orme *et al*. (2012)

Figure 3 – The input phylogeny (λ=1), and a transformed phylogeny (λ=0.4) which constitutes less phylogenetic dependency because shorter internal branches makes all tips more equidistant.

It is necessary to determine if and how phylogeny is related to the variance in the data (predictor and response). In a phylogenetic generalized least square analysis (PGSL; Orme *et al.* 2012) we can construct a covariance matrix that translates the phylogenetic relatedness (separating branch lengths) into expected levels of data covariance between all individual tips. We assume a Brownian motion model of evolution, where the covariance (in trait value) between taxa is solely predicted by the length of the branches separating those taxa. The initial covariance matrix is based on the input tree and thus assumes total phylogenetic dependency in the data, which is expressed as a Pagel's lambda value (λ) of 1 (see Figure 3, λ=1). The next step is to adjust the

covariance matrix in order to increase the fit between the data predicted by the covariance matrix (that assumes Brownian motion) and the actual empirical data. We change the level of phylogenetic dependency by transforming the covariance matrix, which is achieved by multiplying all internal branch lengths by the parameter lambda (λ). The effect of change in λ is illustrated in figure 3. A decrease in internal branch lengths across the phylogeny puts all tips closer to one another (see figure 3, λ=0.4). If this transformation fits the data better, we have less phylogenetic dependency than assumed by the initial covariance matrix. With a λ-value of 0, all internal branch lengths are set to 0, which basically makes all tips phylogenetically equidistant and thus completely phylogenetically independent in a comparative analysis.

Using maximum likelihood, λ is set to the value that makes the phylogenetic covariance matrix best fit the empirical data (due to how the covariance matrix functions, λ can not be greater than 1). At this point we have described the level of phylogenetic dependency that is present in our data. The last step is to take this information from λ and transform the response and explanatory variables in a manner that renders them independent of phylogeny. These transformed data now contains the residual variance after removing the effect of phylogeny and can be analyzed in a normal GLS framework.

Inferring phylogeny – developments in data and technology

Phylogenetics is a biological discipline that aims to describe how biological taxa, e.g. species, are related to one another. Initially, biologists focused on morphological similarities when trying to infer phylogenetic relationships among taxa (Linnaeus 1758). After the emergence of DNA sequencing, however, analysis of homologous genetic sequences has been the common method for inferring phylogeny (Vandamme 2009). Until recently, DNA sequencing was a laborious and time consuming affair, which meant that relatively few genes were used in phylogenetic analysis (Schuster 2008). There was a consensus as to which genes one should analyze in order to answer different questions. For instance, because of their conserved general structure, lack of recombination and high rate of mutations (accumulation of differences), mitochondrial genes were seen as optimal for inferring phylogeny among closely related taxa (Hurst and Jiggins 2005). Single marker analyses have been widely used to infer phylogeny, but there are two fundamental problems this. First, the phylogenetic history of a single gene may differ from the phylogenetic history of the species (Lee *et al.* 2012). The

second problem lies in incomplete lineage sorting, which occurs when too few differences have accumulated among taxa (and internal nodes in the inferred phylogenetic tree; Funk and Omland 2003). Both of these problems can be circumvented by analyzing multiple loci.

With the advent of next-generation sequencing technologies, the availability of genetic data has increased dramatically (Schuster 2008). This sequencing revolution led researchers to expect a similar revolution in the field of phylogenetics (McCormack *et al.* 2013). Sadly, next-gen data has not quite delivered. Some reasons for this are outlined by McCormack *et al.* (2013): 1) Phylogeneticists often work with non-model organisms and 2) there is little consensus as to how libraries (DNA to be sequenced) should be constructed. 3) Unlike in other fields, phylogenetic studies require a large number of individuals per study, and 4) this type of sequencing is still quite costly. Next-gen sequencing is characterized by short sequence reads and data filtering often focuses of retrieving single nucleotide polymorphisms (SNPs). A short stretch of DNA with one single variable position contains very little information (McCormack *et al.* 2013), and in a data set of many such SNPs the relationship between all data points (level of linkage) is unknown. Compared to data where several variable loci are on the same stretch of DNA, the collective amount of information per number of variable loci is low in such data. All of these shortcomings, together with the problem of results not being easily reproducible, make such methodology far from optimal. Additionally, analyzing large amounts of next-gen data is difficult because the analytical algorithms that are available (and considered solid) were designed to analyze a small number of loci. There are alternatives that can handle very large amounts of data, but these still require access to computing clusters and weeks rather than days of computation time. In the early days of "phylogenomics" there were certain analytical tools/approaches that has later been shown to produce flawed results (Philippe *et al.* 2011). Hopefully, the current cutting edge analytical software will survive scrutiny and be further developed.

Having some experience with inferring phylogenies from both single-locus genes and RAD tag SNP data I am not convinced that either of these are the future of phylogenetics. One might think that complete genome sequencing would be optimal, once computational tools/power catches up, but the idea that all loci have a phylogenetic signal is flawed (Hillis and Huelsenbeck 1992). In my opinion, quality should go before quantity in phylogenetic studies. The scientific community has been able to reach consensus on standardization before,

e.g. the barcoding initiative (Hebert *et al.* 2003), and should be able to do it again. Optimally, a multitude of loci of decent length, high variability, excellent reproducibility (primer sequence conservation) between taxa should be identified. By using PCR chip technology with the appropriate primer set, this particular set of loci could easily be amplified and sequenced on a long-read next-gen sequencing platform. By amplifying and sequencing a specific target set of loci, one will not squander sequencing power on uninteresting loci and hence get excellent read depth for the loci in question. Increased read depth makes pooling of individuals from populations feasible, thus reducing costs. If such a protocol was to become the norm, the resulting data could 1) be analyzed by tried and trusted analytical platforms and 2) all analyses following this template would be comparable and reproducible.

RESULTS AND DISCUSSION

Paper I - Female promiscuity is positively associated with neutral and selected genetic diversity in passerine birds

In the first paper we aimed to evaluate whether female promiscuity is correlated with genetic diversity, which has previously been shown by Petrie *et al.* (1998). We examined three different types of markers separately in order to identify whether the association was consistent on different loci and between genomic regions. First, we tested for associations between female promiscuity and intron diversity at autosomal loci and Z-linked (genes on avian sex chromosome Z) loci separately. Female promiscuity was significantly and positively associated with autosomal diversity, but was not associated with Z-linked diversity. The positive association between neutral genetic diversity on autosomal loci is consistent with a hypothesis of compatible genes, i.e. female promiscuity generates genetic diversity because heterozygosity is linked to increased offspring fitness (Foerster *et al.* 2003; García-Navas *et al.* 2009; Olano-Marin *et al.* 2011). We were not able to offer an explanation as to why there was no association between female promiscuity and Z-linked genetic diversity, apart fror the loci involved in cryptic female choice are likely restricted to autosomal chromos Second, we tested for correlation between the number of MHC alleles and female promis The peptide-binding sites on the MHC molecule is the part of the molecule that physically binds to peptide fragments from pathogens, which are further presented to T-cells so that an immune response can be initiated (Janeway *et al.* 2001). These sites are hence expected to be under strong selection compared to the non-peptide binding sites. Our analyses found that female promiscuity predicted the number of alleles at peptide-binding sites, but not at the non-binding sites, i.e. female promiscuity is associated with phenotypic diversity of the operating immune system. That neutral intronic diversity is positively correlated with female promiscuity supports the idea that female promiscuity enables dissasortative mating. If heterozygosity increases fitness in offspring, then the alleles targeted under dissasortative mating are "compatible genes". Based on the positive association between female promiscuity and the number of alleles at peptide-binding sites in the MHC, we hypothesize that female promiscuity is driven by pathogen-mediated selection, where disassortatively mating females perform better when virulent pathogens are present in the population, because their offspring will have increased immunocompetence.

Paper II - Promiscuity, sexual selection, and genetic diversity: A reply to Spurgin

Dr. Lewis G. Spurgin authored a comment on Paper I, which we in turn answered. I will attempt to summarize his three main concerns and give our responses point by point.

- Spurgin points out that our analyses do not take into account the enormous amount of variation in genetic diversity present within species. Our methodology of only sampling subpopulations will in his opinion give an incorrect representation of the genetic variation which is present in species as a whole. Spurgin goes on to state that our sampling strategy would be satisfactory if "promiscuity varied to the same extent as, and strongly correlated with, intraspecific variation in genetic diversity", and points out that we have provided no evidence for this.

Our analyses of genetic diversity were done at the population level, and the level of genetic diversity in the species complexes in their entirety are not a part of our predictions. Spurgin is correct in his assessment that treating the mean trait values from single populations as representative for species is a poor practice with inherent problems. However, we do not treat these estimates of genetic diversity from populations as representative for the species. This is illustrated by using data from two populations of the same species (*Cyanistes caeruleus*) as independent data points in our analyses. The data on female promiscuity, which, much like genetic diversity, may vary between populations of the same species (Petrie and Kempenaers 1998; Griffith *et al.* 2002), were collected from the same populations as the genetic diversity estimates came from. That genetic diversity may vary between populations in a species is not only inconsequential for our methodology, but also supportive of our hypothesis, given that female promiscuity also varies between populations.

- Spurgin describes the correlation between female promiscuity and genetic diversity as surprising because of "the ca. 60 million year evolutionary history of passerine birds, a myriad of mutational, demographic, and selective forces will have altered patterns of genetic diversity within and across species". Spurgin criticizes us for not taking these factors into account in our analyses or discussion.

We acknowledge that many other factors apart from female promiscuity can strongly influence genetic diversity. However, we present an objective statistical test of whether female promiscuity can explain a proportion of the variance in genetic diversity. Given that we only test for a correlation between female promiscuity and genetic diversity, we have no way of

supporting our proposed causality, i.e. that female promiscuity is a driver of genetic diversity. Regardless of these methodological shortcomings, the association is present, statistically supported and warrants interpretation. Our interpretation does not negate the possibility that other evolutionary or ecological factors are important in shaping variation in genetic diversity among populations and species.

- Spurgin deems our dataset to be inadequate (19 populations and 10 introns) for answering the questions we are posing, and states that the interpretation of our results are difficult as a consequence. Spurgin acknowledges that two independent studies have detected a relationship between female promiscuity and genetic diversity, but offers Type I error as the only possible explanation given the unsound assumptions upon which the studies are based.

If we are to explore these associations between female promiscuity and genetic diversity fully, analyses of more comprehensive datasets (species/populations, and loci) are indeed advisable. Analyzing our limited dataset, we found that female promiscuity was significantly associated with both MHC allelic diversity and autosomal intron diversity. When we include the findings of Petrie *et al.* (1998), the hypothesis that genetic diversity and female promiscuity covary has significant support. Type I error is always a possibility, but statistically unlikely to occur in several parallel analyses on non-overlapping datasets. Apart from Type I error, Spurgin does not offer an alternative explanation for these collective results.

Paper III - Migration distance is positively associated with sex-linked genetic diversity in passerine birds

Seasonal migration has been proposed to be associated with female promiscuity in birds (Spottiswoode and Møller 2004). If there is collinearity between migration distance and female promiscuity, migration distance may be a potential confounding variable in the association between female promiscuity and genetic diversity reported in Paper I. We found that female promiscuity significantly explained autosomal genetic diversity regardless of whether migration distance was included in the model. Migration distance was not related to autosomal diversity, but explained variation in genetic diversity on the avian sex chromosome (Z), which, as reported in Paper I, is unrelated to female promiscuity. The finding of association between migration distance and Z-linked genetic diversity is discussed in light of previous hypotheses that outline potential causal links between migration and genetic diversity

(Fitzpatrick 1994; Møller and Erritzøe 1998; Spottiswoode and Møller 2004; Møller *et al.* 2011; Jenkins *et al.* 2012). However, none of these hypotheses fit the observed pattern of an association specific to Z-linked loci. We present a hypothesis that may explain the pattern, namely that sedentary birds have stronger population structuring, and hence smaller population sizes. Smaller population size is expected to negatively affect genetic diversity, through increased drift, on Z-linked loci more so than on autosomal loci (Pool and Nielsen 2007). Migratory species by comparison, who have more gene flow and thus less population structuring (Arguedas and Parker 2000; Rockwell and Barrowclough 1987), will have more genetic diversity (Wang and Caballero 1999).

Paper IV - Resolution of an enigmatic avian island radiation by genome-wide marker analyses

In the fourth paper, we wanted to test whether female promiscuity differed between closely related populations, and whether these differences were explained by phylogenetic relationships. The two study species were African blue tit, represented by the Moroccan and all the Canary Islands populations, and the European blue tit, represented by the Norwegian population. A Levene's test (for equality of variances) revealed a tendency ($p=0.064$) for differing variance in sperm length among populations (figure 4), indicating different levels of female promiscuity. We used the coefficient of among-male variance in sperm length, abbreviated CVbm, as an index of female promiscuity (Kleven et al. 2008b; Lifjeld et al. 2010). CV_{bm} values for our populations are plotted in red in figure 4. Insular island populations are generally characterized by lower levels of female promiscuity than equivalent mainland populations (Griffith 2000). Our results point in the opposite

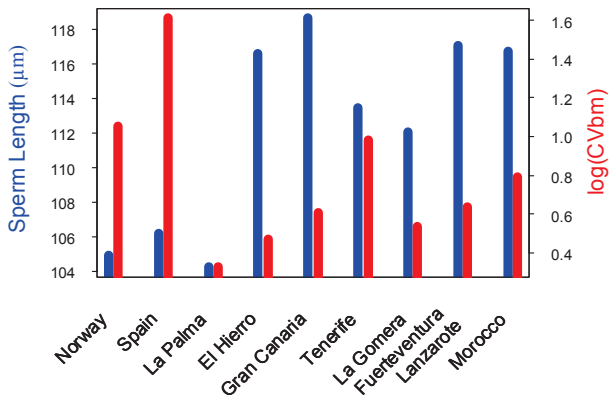


Figure 4 –Average sperm length (blue) and the coefficient of among-male variance in sperm length (CV_{bm}; red).

direction of this prediction, i.e. female promiscuity is more pronounced on islands. Spain had the largest CV_{bm} value, followed by Norway and Teneriffe. The overall picture indicates that there are higher levels of female promiscuity in the African blue tit than in the European blue tit. In order to test whether female promiscuity was constrained by phylogeny, we need to construct a dependable phylogeny. This was done using a mitochondrial gene (COI) and also SNP data from RAD tag sequencing. Our mitochondrial phylogeny reflected a similar overall result as in previous studies, namely that the currently acknowledged African blue tit, which resides on the Canary Islands and in Morocco, is monophyletic. We also constructed two phylogenies from two non-overlapping datasets of one thousand SNPs each. In these analyses, La Palma was placed as sister to Norway. We propose that the inconsistency between the phylogenetic topologies inferred from mitochondrial markers and nuclear SNPs are due to mitochondrial introgression.

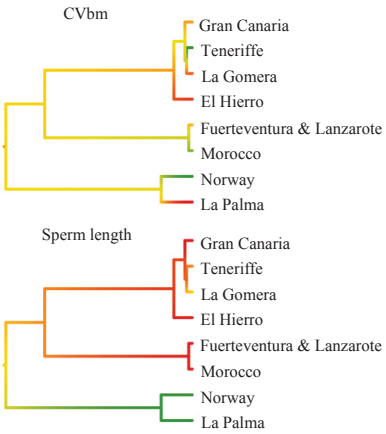


Figure 5 – CV_{bm} and average sperm length values reconstructed and mapped on the phylogeny inferred from the RAD tag SNP data. Sperm length is dependent on phylogeny, while CVbm is not. Green values indicate short sperm lengths and high CV_{bm} (low female promiscuity), and red vice versa.

Using the SNP phylogeny, we estimated the phylogenetic dependency of CV_{bm} to be $\lambda=0$ (likelihood ratio tests of $\lambda=0$, $p=1$ and $\lambda=1$, $p<0.003$). Thus, our results indicate that female promiscuity is different between populations and not dependent on phylogeny (in this dataset). Female promiscuity has been shown to be correlated with sperm length (Kleven *et al.* 2009; Lüpold *et al.* 2009), which we wanted to test using this study system. We found no association between sperm length and CV_{bm} (figure 4) in a PGLS analysis (adjusted R^2=-0.16, $t=0.139$, $p=0.89$, $\lambda=0$). Additionally, tests revealed that, while CV_{bm} was independent of phylogeny, sperm length was strongly dependent on phylogeny ($\lambda=0.84$, likelihood ratio tests of $\lambda=0.03$, $p=1$ and $\lambda=1$, $p<0.14$). This is illustrated by figure 5, where CV_{bm} values and sperm length values are plotted on the phylogeny. Based on high λ-value of sperm length, we postulate that sperm length contain useful phylogenetic information in this study system. In fact, the

distribution in sperm morphology supported the deepest split in the SNP phylogeny. We found that population specific sperm length estimates (figure 4, blue bars) were clustered into two main groups. One group included Norway and La Palma, while the other group included the remaining Canary Islands and Morocco. Both populations in the first group were significantly different from the populations in the second group, but not significantly different from one another. We conclude that there are differences in female promiscuity among populations of African blue tit and European blue tit, and that these differences are not constrained or explained by phylogenetic relationships. We further suggest that sperm length may carry a phylogenetic signal in passerine birds and that a revision of the *Cyanistes* taxonomy may be in needed.

Paper V - Female promiscuity in passerine birds is dependent on phylogeny and associated with male parental care and diet

The fifth and last paper is a comparative study of female promiscuity, where we analyzed data from 95 passerine species, representing 27 families. Passeriformes is an avian order with high levels of female promiscuity (Westneat and Sherman 1997), relatively little variation in life history traits (Owens and Bennett 1995), and altricial young that are dependent on parental care. We found that there was a large degree of phylogenetic dependency in our proxy for female promiscuity (CV_{bm}), which indicates that closely related species are similar with regard to their frequency of female promiscuity. Hence, substantial variation in female promiscuity will be nested among groups such as families. We tested the prediction that variation in female promiscuity at this phylogenetic level would correlate with life history traits, which has been shown in a phylogenetically broader dataset (Arnold and Owens 2002), but found no association. Male parental care is a know covariate of female promiscuity (again shown using broader phylogenetic datasets; Møller 2000; Arnold and Owens 2002) which we found to be significantly and negatively associated with female promiscuity also in our strictly passerine dataset. This association may stem from males reducing their level of parental care in response to loss of within-brood paternity, which could select for less promiscuous behaviour in females. If reductions in male parental care are to function as punishment of promiscuous females, and hence a constraint of female promiscuity, it must simultaneously increase lifetime fecundity in the males (Mauck *et al.* 1999). This latter seems more likely to be the case for long-lived species, such as for instance tubenoses (Procellariiformes), who have low

annual fecundity and reproduce over many seasons. An alternate causality for this association is where male parental care is determined by the availability of extra-pair copulations, i.e. the level of female promiscuity affects male parental care. Based on our result in Paper I, we hypothesized that pathogens/parasites would mediate selection for increased female promiscuity. This was tested by regressing female promiscuity on parasite species richness estimates from both helminths and heamosporidians, which revealed no associations. This negative result may be because we have not analyzed the type of pathogen that is most important for host fitness. We found that the amount of animal tissue in the diet of birds, which is a potential proxy of parasite exposure (Slifko *et al.* 2000), was significantly associated with female promiscuity. The test of collinearity between parasite richness and diet was non-significant. Our results show that while life history is unimportant for female promiscuity in passerines, male parental care and diet explains a significant amount of variance. Regardless, a large amount of variance in female promiscuity in passerines remains unexplained.

CONCLUDING REMARKS

Sex-differences in optimal reproductive strategy cause sexual selection (selection that optimizes reproductive success). One strategy that may optimize the female's reproductive success is mating with males other than the social male (extra-pair copulations) and choosing the sperm that carries the best genes (cryptic female choice). Such female promiscuity is expected to results in antagonism between the sexes (pair-mates) by forcing the social male into reproductive competition with other males. The sexual antagonism may result in male-mediated constraints on female promiscuity.

In this thesis I have examined a major potential benefit of female promiscuity to females, namely compatible genes, which generates genetic diversity in populations (Paper I), while controlling for a proposed covariate, namely migration distance (Paper III). Female promiscuity was associated with higher levels of neutral genetic diversity. The correlation between female promiscuity and neutral genetic diversity was tested at both autosomal and Z-linked loci, but the association was restricted to autosomes. Migration distance, however, was unrelated to autosomal genetic diversity, but was significantly correlated with Z-linked genetic diversity (Paper III). More female promiscuity was also associated with a larger number of alleles involved in pathogen recognition at the MHC, which indicates that promiscuous species have more immunocompetent offspring (Paper I). Given these results, and assuming that there are no costs associated with female promiscuity, one would expect that all species/populations would adopt this strategy. There are however large differences in the frequency of female promiscuity in passerine birds, and these differences may be explained by either benefits or constraints.

In Paper IV I showed how female promiscuity is variable even among very closely related populations and that variation at this level is not restricted by phylogenetic relationships. In a larger dataset (Paper V), I identified a strong phylogenetic signal in female promiscuity, indicating that substantial variance in female promiscuity is nested among families. These collective results show that female promiscuity varies both at the species and family level. Different variables will likely be associated with female promiscuity at different levels of classification (Griffith *et al.* 2002). In the larger dataset (Paper V), female promiscuity was positively associated with diet, a potential proxy for parasite exposure, and negatively associated with male parental care. Male parental care may either constitute a

constraint on promiscuous behaviour in females or be determined by the availability of extra-pair copulations, i.e. the causal direction between female promiscuity and male parental care is unknown.

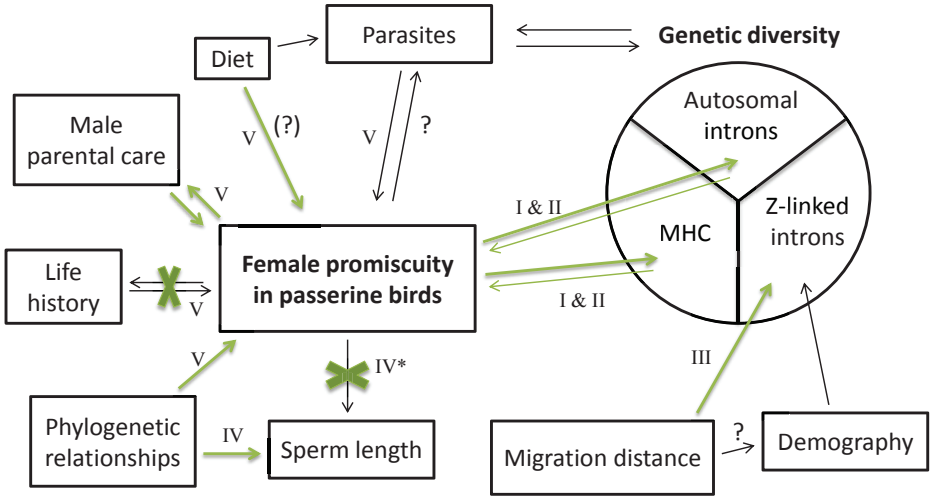


Figure 6 – A flow chart showing possible causal links and associations between the variables I have discussed in my dissertation. Green arrows show associations with empirical support, with thick arrows indicating proposed causal direction. Black arrows represent hypothesized associations or causal pathways where I have not provided empirical support. Green Xs represents rejection of hypotheses for particular datasets. Question marks indicate untested hypotheses or tested associations with lacking support. Black arrows with no question marks have support in the literature. Roman numerals refer to which papers treat the respective relationships. *This relationship was not discussed in the manuscript corresponding to Paper IV, but is included in the Results and Discussion section of this synthesis.

My contribution to the field of female promiscuity in passerine birds is reviewed in figure 6. The observant reader will notice some question marks, which indicate unexplored hypotheses or areas where I failed to provide empirical support. This, in addition to the large amount of variance in female promiscuity that remains unexplained, clearly shows that there is more work to be done in this field. We should of course try to identify other covariates of female promiscuity, but comparative methodology may also be useful for further exploring the associations described in this dissertation. By collecting data of higher quality for a smaller number of species we can, for instance, determine whether diet is associated with the slighter

differences in female promiscuity also among closely related species, and also get a better estimate of how much variance in female promiscuity is explained by diet. By applying next-gen sequencing we can get more data on neutral genetic diversity and get better coverage of the number of MHC alleles and thus better test how female promiscuity is associated with genetic diversity. If we could determine how important pathogens are to bird fitness, i.e. quantifying pathogen-related mortality, the pathogen-mediated selection hypothesis could be retested in a comparative framework. Generally, datasets of high quality/detail will be difficult to collect, but by focusing on a small number of carefully chosen species that exhibit considerable variation in female promiscuity and little variation in potential confounding variables, comparative analyses can be very informative.

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LITERATURE

Aquadro, C. F. 1992. Why is the genome variable? Insights from Drosophila. Trends in Genetics 8:355-362.

Arct, A., S. Drobniak, E. Podmokła, L. Gustafson, and M. Cichoń. 2013. Benefits of extra-pair mating may depend on environmental conditions—an experimental study ıe blue tit (*Cyanistes caeruleus*). Behav. Ecol. Sociobiol. 67:1809-1815.

Arguedas, N., and P. G. Parker. 2000. Seasonal migration and genetic population structure in house wrens. Condor 102:517-528.

Arnold, K. E., and I. P. F. Owens. 2002. Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. Proc. R. Soc. B 269:1263-1269.

Bengtsson, B. O. 2003. Genetic variation in organisms with sexual and asexual reproduction. J. Evol. Biol. 16:189-199.

Bernstein, H., and C. Bernstein. 2010. Evolutionary Origin of Recombination during Meiosis. BioScience 60:498-505.

Brouwer, L., I. Barr, M. Van De Pol, T. Burke, J. A. N. Komdeur, and D. S. Richardson. 2010. MHC-dependent survival in a wild population: evidence for hidden genetic benefits gained through extra-pair fertilizations. Mol. Ecol. 19:3444-3455.

Burke, T., N. B. Davies, M. W. Bruford, and B. J. Hatchwell. 1989. Parental care and mating behaviour of polyandrous dunnocks (*Prunella modularis*) related to paternity by DNA fingerprinting. Nature 338:249-251.

Clutton-Brock, T. H., F. E. Guinness, and S. D. Albon. 1982. Red deer: Behavior and ecology of the two sexes. University of Chicago Press, Chicago.

Dietzen, C., E. Garcia-del-Rey, G. D. Castro, and M. Wink. 2008. Phylogeography of the Blue Tit (*Parus teneriffae*-group) on the Canary Islands based on mitochondrial DNA sequence data and morphometrics. J. Ornithol. 149:1-12.

Dunn, P. O., J. L. Bollmer, C. R. Freeeman-Gallant, and L. A. Whittingham. 2013. MHC variation is related to a sexually selected ornament, survival and parasite resistance in common yellowthroats. Evolution 67:679-687.

Elgar, M., T. Jones, and K. McNamara. 2013. Promiscuous words. Frontiers in Zoology 10:66.

Fitzpatrick, S. 1994. Colorful migratory birds - Evidence for a mechanism other than parasite resistance for the maintenance of good genes sexual selection. Proc. R. Soc. B 257:155-160.

Foerster, K., K. Delhey, A. Johnsen, J. T. Lifjeld, and B. Kempenaers. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714-717.

Fossøy, F., A. Johnsen, and J. T. Lifjeld. 2008. Multiple genetic benefits of female promiscuity in a socially monogamous passerine. Evolution 62:145-156.

Frankham, R. 2005. Genetics and extinction. Biological Conservation 126:131-140.

Funk, D. J., and K. E. Omland. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. S. 34:397-423.

García-Navas, V., J. Ortego, and J. J. Sanz. 2009. Heterozygosity-based assortative mating in blue tits (*Cyanistes caeruleus*): implications for the evolution of mate choice. Proc. R. Soc. B 276:2931-2940.

Grant, P. R. 1979. Ecological and morphological variation of Canary Island blue tits, *Parus caeruleus* (Aves: Paridae). Biol. J. Linn. Soc. 11:103-129.

Griffith, S. 2000. High fidelity on islands: a comparative study of extra-pair paternity in passerine birds. Behav. Ecol. 11:265-273.

Griffith, S. C., I. P. F. Owens, and K. A. Thuman. 2002. Extrapair paternity in birds: a review of interspecific variation and adaptive function. Mol. Ecol. 11:2195-2212.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol. Evol. 11:92-98.

Gwynne, D. T. 1991. Sexual competition among females: What causes courtship-role reversal? Trends Ecol. Evol. 6:118-121.

Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (a review). Proc. Natl. Acad. Sci. 87:3566-3573.

Harvey, P. H., and M. Pagel. 1991. The Comparative Method in Evolutionary Biology. Oxford Univ. Press, Oxford.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. DeWard. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. B 270:313-321.

Hillis, D. M., and J. P. Huelsenbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. Journal of Heredity 83:189-195.

Hosmer, D. W., and S. Lemeshow. 2005. Applied Logistic Regression. John Wiley & Sons, Inc., New Jersey.

Hurst, G. D. D., and F. M. Jiggins. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. R. Soc. B 272:1525-1534.

Janeway, C. A., J. R. P. Travers, M. Walport, and M. J. Shlomchik. 2001. Antigen Presentation to T Lymphocytes. Immunobiology -The Immune System in Health and Disease. Garland Science, New York.

Jenkins, T., G. H. Thomas, O. Hellgren, and I. P. F. Owens. 2012. Migratory behavior of birds affects their coevolutionary relationship with blood parasites. Evolution 66:740-751.

Johnsen, A., V. Andersen, C. Sunding, and J. T. Lifjeld. 2000. Female bluethroats enhance offspring immunocompetence through extra-pair copulations. Nature 406:296-299.

Johnsen, A., J. T. Lifjeld, P. A. Rohde, C. R. Primmer, and H. Ellegren. 1998. Sexual conflict over fertilizations: female bluethroats escape male paternity guards. Behav. Ecol. Sociobiol. 43:401-408.

Kempenaers, B., B. Congdon, P. Boag, and R. J. Robertson. 1999. Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatitbility hypothesis? Behav. Ecol. 10:304-311.

Kleven, O., B.-A. Bjerke, and J. T. Lifjeld. 2008a. Genetic monogamy in the common crossbill (*Loxia curvirostra*). J. Ornithol. 149:651-654.

Kleven, O., F. Fossøy, T. Laskemoen, R. J. Robertson, G. Rudolfsen, and J. T. Lifjeld. 2009. Comparative evidence for the evolution of sperm swimming speed by sperm competition and female sperm storage duration in passerine birds. Evolution 63:2466-2473.

Kleven, O., T. Laskemoen, F. Fossøy, R. J. Robertson, and J. T. Lifjeld. 2008b. Intraspecific variation in sperm length is negatively related to sperm competition in passerine birds. Evolution 62:494-499.

Kvist, L., J. Broggi, J. C. Illera, and K. Koivula. 2005. Colonisation and diversification of the Blue Tits (*Parus caeruleus teneriffae*-group) in the Canary Islands. Mol. Phyl. Evol. 34:501-511.

Lack, D. 1968. Ecological adaptations for breeding in birds. Methuen, London.

Lee, J. Y., L. Joseph, and S. V. Edwards. 2012. A Species Tree for the Australo-Papuan Fairy-wrens and Allies (Aves: Maluridae). Systematic Biology 61:253-271.

Lifjeld, J. T., T. Laskemoen, O. Kleven, T. Albrecht, and R. J. Robertson. 2010. Sperm length variation as a predictor of extrapair paternity in passerine birds. PLoS ONE 5:e13456.

Linnaeus, C. 1758. Systema Naturae, Stockholm.

Lodé, T. 2012. Sex and the origin of genetic exchanges. Trends in evolutionary biology 4:e1.

Lüpold, S., S. Calhim, S. Immler, and T. R. Birkhead. 2009. Sperm morphology and sperm velocity in passerine birds. Proc. R. Soc. B 276:1175-1181.

Matysioková, B., and V. Remeš. 2013. Faithful females receive more help: the extent of male parental care during incubation in relation to extra-pair paternity in songbirds. J. Evol. Biol. 26:155-162.

Mauck, R. A., E. A. Marschall, and P. G. Parker. 1999. Adult survival and imperfect assessment of parentage: effects on male parenting decisions. Am. Nat. 154:99-109.

McCormack, J. E., S. M. Hird, A. J. Zellmer, B. C. Carstens, and R. T. Brumfield. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Mol. Phyl. Evol. 66:526-538.

Morell, V. 1998. A new look at monogamy. Science 281:1982-1983.

Mulder, R. A., P. O. Dunn, A. Cockburn, K. A. Lazenby-Cohen, and M. J. Howell. 1994. Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. Proc. R. Soc. B 255:223-229.

Møller, A., and J. Erritzøe. 1998. Host immune defence and migration in birds. Evolutionary Ecology 12:945-953.

Møller, A. P. 1988. Female choice selects for male sexual tail ornaments in the monogamous swallow. Nature 332:640-642.

Møller, A. P. 2000. Male parental care, female reproductive success, and extrapair paternity. Behav. Ecol. 11:161-168.

Møller, A. P., L. Z. Garamszegi, J. M. Peralta-Sánchez, and J. J. Soler. 2011. Migratory divides and their consequences for dispersal, population size and parasite–host interactions. J. Evol. Biol. 24:1744-1755.

Olano-Marin, J., J. C. Mueller, and B. Kempenaers. 2011. Heterozygosity and survival in blue tits (*Cyanistes caeruleus*): contrasting effects of presumably functional and neutral loci. Mol. Ecol. 20:4028-4041.

Oring, L., and D. B. Lank. 1982. Sexual selection, arrival times, philopatry and site fidelity in the polyandrous spotted sandpiper. Behav. Ecol. Sociobiol. 10:185-191.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2012. Comparative analyses of phylogenetics and evolution in R. R package version 0.5.

Owens, I. P. F., and P. M. Bennett. 1995. Ancient ecological diversification explains life-history variation among living birds. Proc. R. Soc. B 261:227-232.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in insects. Biol. Rev. 45:525-567.

Parker, G. A. 2006. Sexual conflict over mating and fertilization: an overview. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 361:235-259.

Petrie, M., C. Doums, and A. P. Møller. 1998. The degree of extra-pair paternity increases with genetic variability. Proc. Natl. Acad. Sci. 95:9390-9395.

Petrie, M., and B. Kempenaers. 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends Ecol. Evol. 13:52-58.

Philippe, H., H. Brinkmann, D. V. Lavrov, D. T. J. Littlewood, M. Manuel, G. Wörheide, and D. Baurain. 2011. Resolving difficult phylogenetic questions: Why more sequences are not enough. PLoS Biol 9:e1000602.

Pool, J. E., and R. Nielsen. 2007. Population size changes reshape genomic patterns of diversity. Evolution 61:3001-3006.

Promerová, M., M. Vinkler, J. Bryja, R. Poláková, J. Schnitzer, P. Munclinger, and T. Albrecht. 2011. Occurrence of extra-pair paternity is connected to social male's MHC-variability in the scarlet rosefinch *Carpodacus erythrinus*. J. Avian Biol. 42:5-10.

Pusey, A. E., and C. Packer. 1994. Infanticide and Parental Care. Harwood Academic Press, Chur, Switzerland.

Päckert, M., J. Martens, J. Hering, L. Kvist, and J. C. Illera. 2013. Return flight to the Canary Islands – The key role of peripheral populations of Afrocanarian blue tits (Aves: *Cyanistes teneriffae*) in multi-gene reconstructions of colonization pathways. Mol. Phyl. Evol. 67:458-467.

Reed, D. H., and R. Frankham. 2003. Correlation between Fitness and Genetic Diversity. Conservation Biology 17:230-237.

Richardson, D. S., J. Komdeur, T. Burke, and T. von Schantz. 2005. MHC-based patterns of social and extra-pair mate choice in the seychelles warbler. Proc. R. Soc. B 272:759–767.

Rockwell, R. F., and G. F. Barrowclough. 1987. Gene flow and the genetic structure of populations. *in* F. Cooke, and P. A. Buckley, eds. Avian genetics, a population and ecological approach. Academic Press, San Diego, CA.

Schuster, S. C. 2008. Next-generation sequencing transforms today's biology. Nat Meth 5:16-18.

Sheldon, B. C. 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. Proc. R. Soc. B 257:25-30.

Slifko, T. R., H. V. Smith, and J. B. Rose. 2000. Emerging parasite zoonoses associated with water and food. International Journal for Parasitology 30:1379-1393.

Spottiswoode, C., and A. P. Møller. 2004. Extrapair paternity, migration, and breeding synchrony in birds. Behav. Ecol. 15:41-57.

Tregenza, T., and N. Wedell. 2000. Genetic compatibility, mate choice and patterns of parentage: Invited Review. Mol. Ecol. 9:1013-1027.

Vandamme, A. 2009. Basic consepts of molecular evolution *in* P. Lemey, M. Salemi, and A. Vandamme, eds. The Phylogenetic Handbook. Cambridge University Press, New York.

Vos, M. 2009. Why do bacteria engage in homologous recombination? Trends in Microbiology 17:226-232.

Wan, D., P. Chang, and J. Yin. 2013. Causes of extra-pair paternity and its inter-specific variation in socially monogamous birds. Acta Ecologica Sinica 33:158-166.

Wang, J., and A. Caballero. 1999. Developments in predicting the effective size of subdivided populations. Heredity 82:212-226.

Westneat, D. F., and P. W. Sherman. 1997. Density and extra-pair fertilizations in birds: a comparative analysis. Behav. Ecol. Sociobiol. 41:205-215.

Westneat, D. F., P. W. Sherman, and M. L. Morton. 1990. The ecology and evolution of extra-pair copulations in birds. Curr. Ornithol. 7:331-369.

Williams, G. C. 1975. Sex and evolution. Princeton University Press, Princeton, New Jersey.

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